Prediagnostic Circulating Antibodies to JC and BK Human Polyomaviruses and Risk of Non-Hodgkin Lymphoma

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Abstract

Viral infections have been associated with increased risk of non–Hodgkin's lymphoma (NHL). We conducted a nested case-control study to investigate the association between prediagnostic serum antibodies to the human polyomaviruses, JC (JCV) and BK (BKV), and subsequent risk of NHL. Two research serum banks were established in Washington County, Maryland, in 1974 and 1989, with the collection of blood samples from >45,000 volunteers. Incident NHL cases diagnosed through 2002 (n = 170) were identified among participants by linkage to population-based cancer registries. Two controls were matched to each case (n = 340) on age, sex, and blood draw date. Prediagnostic IgG antibodies to JCV and BKV were measured using virus-like particle ELISA. Associations between JCV and BKV antibody seropositivity and NHL were estimated using conditional logistic regression. Overall, neither antibodies to JCV [odds ratio (OR), 0.83; 95% confidence interval (95% CI), 0.56-1.23] nor BKV (OR, 0.98; 95% CI, 0.64-1.48) were associated with an increased risk of NHL. Results were similar after stratification by NHL subtype or induction period and adjustment for EBV seropositivity. Among those who donated blood in both 1974 and 1989, an increase in JCV antibody levels over time was associated with a 4-fold increased risk of NHL compared with a steep decline in antibody levels (OR, 4.59; 95% CI, 1.30-16.25; P_{trend} = 0.02). Whereas JCV seropositivity was not associated with NHL overall, the finding of an increased risk of NHL associated with increasing antibody levels among those who were seropositive at baseline warrants further research into factors influencing reactivation of JCV infection. (Cancer Epidemiol Biomarkers Prev 2006;15(3):543–50)

Introduction

Non–Hodgkin's lymphoma (NHL) is the most common hematologic malignancy in the United States and is predicted to be the sixth and fifth most commonly diagnosed cancer in 2005 among U.S. men and women, respectively (1). The doubling of NHL incidence from the 1970s to 1990s (1) cannot be explained by AIDS-related immunosuppression, and few other risk factors for NHL have been consistently identified. Several viruses are associated with one or more NHL subtypes, including HIV (2) and Epstein-Barr Virus (EBV) (refs. 3, 4).

JC virus (JCV) and BK virus (BKV) comprise the human polyomaviruses and are common viral infections, with antibody seroprevalences among U.S. adults ranging from 44% (5) to 75% (6, 7) for JCV and 63% (5) to 80% (7) for BKV. JCV seroprevalence increases with age throughout adulthood, whereas BKV seroprevalence peaks in early adulthood and declines with subsequent age (5, 7, 8). Initial infections with JCV and BKV are thought to be asymptomatic and usually occur in early childhood (BKV) or later childhood and adolescence (JCV), after which the viruses remain latent in the kidneys (7). Other sites of latency have been proposed, including the brain and B-lymphocytes for JCV (7). Both viruses can reactivate with immunosuppression, resulting in disease. For example, JCV causes progressive multifocal leukoencephalopathy in AIDS patients (9), and BKV is associated with nephropathy in kidney transplant recipients (7). Reactivation of JCV and BKV infections also occurs in individuals without severe immunosuppression, with JCV and BKV detected in 37% to 47% (10-12) and 0% to 5% (11, 12) of urine samples from immunocompetent individuals (10-12), respectively. However, no overt disease associated with reactivation of polyomavirus infection in immunocompetent individuals has yet been identified.

Infections with JCV and BKV have been proposed as potential risk factors for cancer, due in part to the ability of these viruses to induce tumors in experimentally infected laboratory animals (13). Both viruses encode for a nonstructural protein called the large tumor (T) antigen, which initiates viral DNA replication and modulates gene transcription (7). Large T-antigen can also bind to and inactivate the tumor suppressor proteins p53 and pRb (13), inhibiting apoptosis. JCV has been shown to infect B-lymphocytes (14, 15), and Neel and colleagues have described chromosomal damage in lymphocytes associated with both JCV (16, 17) and BKV (16) infections. Some investigators have detected polyomavirus sequences from NHL tissues, including one laboratory that detected JCV and BKV sequences in 5% and 6% of 83 lymphomas, respectively (18) and a recent report that showed the presence of JCV DNA sequences in 22 of 27 B-cell lymphomas of the central nervous system, a subset of which also contained EBV DNA (19).

Although these data suggest a potential role for JCV in NHL, other studies have not detected JCV or BKV sequences in lymphoma tissues, including three studies that primarily investigated the presence of SV40 (20-22). Additionally, a recent multicenter case-control study observed a decreased risk of NHL associated with antibodies to JCV and no association with antibodies to BKV (23). However, antibodies measured at the time of NHL diagnosis may not accurately reflect antibodies that would have been circulating in the early

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stages of lymphomagenesis. To investigate the association between infection with JCV and BKV and the risk of NHL diagnosed up to 30 years later, we conducted a population-based case-control study of serum antibodies to JCV and BKV and NHL nested within the CLUE cohort studies in Washington County, Maryland.

Materials and Methods

Study Population. Two research serum banks were established in Washington County, Maryland, both named ''CLUE'' for the slogan, ''Give us a clue to heart disease and cancer.'' The first CLUE study, CLUE I, was conducted in 1974 with the collection of 23,951 blood specimens from county residents in August through November 1974. Participation was best in the age group 35 to 65 years, and was slightly better among females, the better-educated, and nonsmokers. The second CLUE study, CLUE II, was conducted between May and November 1989 with the collection of 25,080 blood specimens from county residents. Serum (CLUE I) or plasma (CLUE II) was obtained from each participant in addition to brief demographic data. All specimens have been stored at −70°C or colder. All participants gave consent at the time of blood draw for their blood sample to be used in future research. The protocol for the current study was approved by the Committee on Human Research, the institutional review board at the Johns Hopkins Bloomberg School of Public Health.

The cases and controls included in the present study are the same individuals previously investigated for antibodies to the primate polyomavirus, SV40 (24). Cases of NHL occurring among CLUE cohort members through 2002 were identified by linkage to the Washington County Registry [International Classification of Diseases (ICD)-8 code 200 or 202] and, since 1992, the Maryland State Cancer Registry. Cases were defined as participants of one or both of the CLUE cohorts who were Washington County residents at the times of both blood donation and diagnosis with NHL. NHL had to have been the cases' first cancer diagnosis, with the possible exceptions of nonmelanoma skin cancer or cervical cancer *in situ*. NHL subtypes were classified using ICD-9/-10 morphology codes: diffuse large B-cell lymphomas (ICD 9680/3, 9682/3, 9684/3, and 9680), follicular B-cell lymphomas (9675/3, 9690/3, 9691/3, 9695/3, 9696/3, 9698/3, and 9690), T-cell lymphomas (9700/3), and others/not specified (9590/3, 9591/3, 9592/3, 9593/3, 9595/3, 9670/3, 9672/3, 9673/3, 9686/3, 9687/3, 9694/3, 9699/3, 9711/3, 9823/3, 9940/3, 9533/1, 9693/3, and 9685/3). One hundred seventy-four cases were identified, two of which were determined to be Hodgkin's disease and were excluded. Two additional cases were excluded due to inadequate amounts of available serum, yielding a final case sample size of 170. Of these 170 NHL cases, 91 participated in CLUE I only, 32 participated in CLUE II only, and 47 participated in both CLUE I and II, contributing a total of 217 blood samples for polyomavirus antibody analysis.

Two controls were matched to each case on sex, race, age within 1 year, freeze/thaw status of the serum or plasma, and participation in CLUE I, CLUE II, or both. Controls were residents of Washington County at the time of blood donation who were not known to have died or developed cancer (except for possibly nonmelanoma skin cancer or cervical cancer *in situ*) as of the date of diagnosis of the case. Matching criteria were relaxed in certain cases to achieve a match: seven controls were up to 2 years older than their matched cases, and for four case-control pairs, date of blood draw differed as much as 1 month. In accordance with case matching, 182 controls participated in CLUE I only, 64 participated in CLUE II only, and 94 participated in both cohorts, contributing a total of 434 blood samples for polyomavirus antibody analysis.

A subset of cases (n = 87) diagnosed through 1994 and their matched controls (n = 150) were included from a previous study in which an association between prediagnostic EBV antibody seropositivity and NHL was observed (25). Because both latent EBV and polyomavirus infections can reactivate under conditions of immunosuppression to cause disease, and JCV and EBV sequences have been shown to be present in NHL tissues simultaneously (19), we also assessed whether there was an additive effect of EBV and JCV or BKV infection on the risk of NHL in the subset of participants for whom both EBV and polyomavirus antibody data were available. This subset differs slightly from the previous study due to missing EBV data and replacement of controls from the 1994 study who were subsequently diagnosed with cancer or whose banked serum was depleted. In the present study, EBV antibody data were thus available for 96 blood samples from 84 NHL cases and 167 blood samples from 148 controls.

Laboratory Methods. Recombinant baculoviruses expressing the VP1 major capsid protein of JCV or BKV were kindly provided by Stephen Frye and Peter Jensen (Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke, Bethesda, MD) (ref. 26). Insect cells (*sf*9) were infected with the recombinant baculoviruses and virus-like particles were purified from cell lysates by CsCl density gradient ultracentrifugation and cation exchange or gel exclusion liquid chromatography as previously described (27, 28).

Wells of PolySorp microtiter plates (Nunc, Naperville, IL) were coated overnight at 4°C with 20 to 30 ng BKV or JCV virus-like particle protein in PBS (pH 7.2) and blocked for 3 hours at room temperature with 0.5% (w/v) polyvinyl alcohol, MW 30,000 to 70,000 (Sigma, St. Louis, MO), in PBS (0.5% polyvinyl alcohol). Before use and following each incubation step, the plates were washed four times with PBS containing 0.05% (v/v) Tween 20 (Sigma) in an automatic plate washer (Skanwasher 300, Skatron, Lier, Norway). Serum samples diluted 1:400 in 0.5% polyvinyl alcohol were left to react for 1 hour at 37°C. Antigen-bound immunoglobulin was detected with peroxidase-conjugated goat antibodies against human IgG (Zymed, San Francisco, CA), diluted 1:4,000 in 0.5% polyvinyl alcohol 0.0025% Tween 20, 0.8% (w/v) polyvinylpyrrolidone, MW 360,000 (Sigma) in PBS. After 30 minutes at 37°C, color development was initiated by the addition of 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) hydrogen peroxide solution (Kirkegaard and Perry, Gaithersburg, MD). The reaction was stopped after 20 minutes by addition of 1% dodecyl sulfate and absorbance was measured at 405 nm in an automated microtiter plate reader (Molecular Devices, Menlo Park, CA).

Each case and its two matched controls were maintained in their matched triplet (or ''set'') to ensure simultaneous processing. Laboratory personnel were masked as to the case-control status of each sample. Seven pooled serum samples and eight pooled plasma samples were masked and placed across sets to test interset reliability of the ELISA assay. All seven pooled serum samples and all eight pooled plasma samples tested positive for JCV and BKV. Similarly, nine duplicate pairs of sera and eight duplicate pairs of plasma were placed within matched sets to assess intraset reliability, each pair drawn from a cohort member not included in the study. Both samples in all nine duplicate pairs of sera tested positive for JCV, and both samples in eight of nine pairs tested positive for BKV, with both samples in the remaining pair testing negative for BKV. Both samples in each of eight duplicate pairs of plasma tested positive for both JCV and BKV.

EBV antibodies were measured for a nested case-control study of NHL conducted within the CLUE cohorts in 1994 using immunofluoresence assays to measure IgG antibodies to EBV early antigen and viral capsid antigen as previously described (29). Positive early antigen antibodies were defined

as reciprocal titers of ≥20, and reciprocal viral capsid antigen antibody titers of ≥640 were considered to be elevated.

Statistical Methods. CLUE baseline characteristics were compared between cases and controls using the χ^2 test. Geometric mean absorbance values were calculated for cases and controls and compared using a *t* test. Individuals were defined as positive or negative for polyomavirus antibodies using an absorbance value cutpoint of 0.1, as determined from previous results (27). Associations between polyomavirus seropositivity and age at blood draw were assessed separately for controls at two time points using logistic regression, with serostatus as the outcome and age at blood draw as a continuous independent variable. The associations between polyomavirus antibody seropositivity and NHL were estimated by matched odds ratios (OR) calculated using conditional logistic regression. The initial analysis was stratified by cohort participation. ORs were similar using either serum samples collected in 1974 or plasma samples collected in 1989; thus, all samples were combined in further analyses. Forty-seven cases and their matched controls donated blood to both CLUE I and II, and repeated measures of JCV and BKV antibody levels in this subgroup were accounted for in the regression models using robust sandwich estimation (30).

Similar conditional logistic regression models were also used to calculate matched ORs for the association between EBV seropositivity and NHL among the subset of participants for whom EBV antibody data were available. ORs were calculated separately for antibody seropositivity to each viral antigen (JCV virus-like particle, BKV virus-like particle, EBV early antigen, and EBV viral capsid antigen), with and without adjustment for antibody seropositivity to the other viruses.

To investigate differences in risk across NHL subtypes, matched ORs for seropositivity to JCV, BKV, and EBV early antigen were calculated separately for diffuse large B-cell lymphoma, follicular lymphoma, and other/not specified subtypes. To investigate the previously raised hypothesis that JCV may contribute to the earliest stages of NHL development, possibly through a hit-and-run mechanism (23, 31), associations between polyomavirus antibodies and NHL were compared across three 10-year strata of time between blood draw and diagnosis (i.e., hypothetical induction period).

Additional analyses were conducted within the subgroup of CLUE participants who donated blood in both 1974 and 1989. Individuals were first considered positive or negative for polyomavirus antibodies at both time points, using the absorbance value of 0.1 (24). To assess quantitative changes, antibody levels from both time points were plotted against age at blood draw, with a line connecting the antibody levels at the two time points for each individual. These longitudinal plots were generated separately for NHL cases versus controls. To evaluate the risk of NHL associated with quantitative changes in antibody levels over time in the plots, we first calculated the absolute difference in absorbance values between 1974 and 1989 by subtracting the absorbance value in 1974 from the absorbance value in 1989. Thus, negative differences indicated a decrease in antibody levels over time, whereas positive differences indicated an increase over time. Antibody levels to JCV and BKV decreased over time for ~75% of this subgroup, and these negative changes were categorized into thirds, based on the distributions in the controls. Using as a reference group those individuals whose antibody levels declined the greatest, matched ORs were calculated to investigate a trend in risk of NHL associated with change in antibody levels. *P* values for trends were calculated by assigning the median absorbance value for the appropriate category into which each individual fell and including this variable in the regression model.

For the subgroup of participants in the 1994 study, EBV early antigen reciprocal antibody titers were plotted as ordinal values against age at blood draw: reciprocal titers of <10, 10, 20, 40, 80, 160, and 320 were assigned ordinal values of 0, 1, 2, 3, 4, 5, and 6, respectively. To calculate matched ORs for the risk of NHL associated with change in EBV titers, we considered four categories of change in antibodies over time, based on pairwise combinations of being seronegative or seropositive at the two time points (1974 and 1989). There were too few observations to conduct a matched analysis; thus, we estimated unmatched ORs for change in EBV early antigen antibody serostatus using logistic regression, adjusted for age at blood draw, sex, and race (i.e., matching factors).

All statistical tests were two-sided. Analyses were conducted using SAS, version 8 (SAS Institute, Inc., Cary, NC). Longitudinal plots were produced using Stata, version 8.2 (StataCorp, College Station, TX).

Results

Baseline characteristics of NHL cases and controls are presented in Table 1. Greater than half of cases were female, and all but one case was White, reflecting the gender and race distributions of the underlying cohorts. Education and smoking at baseline were not associated with the development of NHL (Table 1). Diffuse large B-cell lymphoma was the most common subtype of NHL (41.2%), follicular lymphomas comprised 25.3% of cases, three cases of T-cell lymphoma were identified (1.8%), and 31.8% of cases were of other or unknown types.

Among controls, JCV seroprevalence increased with age in both 1974 (P = 0.17) and 1989 (P = 0.11), whereas BKV seroprevalence decreased with age in 1974 (P = 0.20) and increased with age in 1989 (P = 0.06). None of these associations were statistically significant. Geometric mean absorbance values did not differ between cases and controls for either JCV antibodies (cases = 0.19, controls = 0.23, P = 0.21) or BKV antibodies (cases = 0.18, controls = 0.17, P = 0.48). A smaller proportion of cases (65%) than controls (68%) was positive for antibodies to JCV, although this difference was not statistically significant [OR, 0.83; 95% confidence interval (95% CI), 0.56-1.23; Table 2]. BKV antibody seroprevalence was equivalent among cases (67%) and controls (67%; OR, 0.98; 95%

Table 1. Baseline characteristics of NHL cases and matched controls, Washington County, Maryland, 1975 to 2002

Characteristics	Cases (N = 170)	Controls (N = 340)
	n (%)	*n* (%)
CLUE participation*		
CLUE I only (1974)	91 (53.5)	182 (53.5)
CLUE II only (1989)	32 (18.8)	64 (18.8)
CLUE I and II	47 (27.6)	94 (27.6)
Gender*		
Female	93 (54.7)	186 (54.7)
Male	77 (45.3)	154 (45.3)
Race		
White	169 (99.4)	337 (99.1)
Black or other	1 (0.6)	3 (0.9)
Age at blood draw (y)*,†		
Mean (SD)		
CLUE I baseline (1974)	50.9 (12.8)	51.0 (12.8)
CLUE II baseline (1989)	61.6 (11.4)	61.5 (11.8)
Education ‡		
<12 grades	67 (39.4)	130 (38.5)
12 grades	62 (36.5)	143 (42.3)
>12 grades	41 (24.1)	65 (19.2)
Smoking status		
Never	75 (44.1)	153 (45.0)
Former	55 (32.4)	110 (32.4)
Current	40 (23.5)	77 (22.7)

*Age, gender, and CLUE participation were matching factors.
†Study subjects who are participants of both CLUE I and II are counted twice.
‡Information on education was missing for two controls.

Table 2. Antibodies to JCV, BKV, and EBV in relation to subsequent risk of NHL among 140 cases and 340 matched controls, Washington County, Maryland, 1975 to 2002

Antibody serostatus (adjusted for)	Clue I cohort (1974)			Clue II cohort (1989)			Clue I and II cohorts combined*		
	Cases, *n* (%)	Controls, *n* (%)	OR† (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR† (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR†,* (95% CI)
All NHL cases									
JCV negative	38 (28)	68 (25)	1.00	39 (49)	70 (44)	1.00	77 (36)	138 (39)	1.00
JCV positive	100 (73)	208 (75)	0.86 (0.53-1.37)	40 (51)	88 (56)	0.79 (0.45-1.41)	140 (65)	296 (68)	0.83 (0.56-1.23)
(BKV)			0.85 (0.53-1.37)			0.76 (0.42-1.37)			
BKV negative	38 (28)	63 (23)	1.00	34 (43)	79 (50)	1.00	72 (33)	142 (33)	1.00
BKV positive	100 (73)	213 (77)	0.77 (0.47-1.24)	45 (57)	79 (50)	1.38 (0.77-2.47)	145 (67)	292 (67)	0.98 (0.64-1.48)
(JCV)			0.76 (0.47-1.24)			1.42 (0.79-2.56)			
Cases diagnosed through 1994									
JCV negative	13 (18)	24 (18)	1.00	4 (17)	10 (29)	1.00	17 (18)	34 (20)	1.00
JCV positive	60 (82)	108 (82)	1.06 (0.47-2.39)	19 (83)	25 (71)	1.78 (0.46-6.94)	79 (82)	133 (80)	1.23 (0.61-2.47)
(EBV EA)			0.99 (0.43-2.28)			1.85 (0.44-7.73)			1.17 (0.58-2.35)
(EBV VCA)			1.02 (0.44-2.33)			1.78 (0.46-6.91)			1.19 (0.59-2.42)
BKV negative	18 (25)	23 (17)	1.00	6 (26)	9 (26)	1.00	24 (25)	32 (19)	1.00
BKV positive	55 (75)	109 (83)	0.67 (0.32-1.34)	17 (74)	26 (74)	1.00 (0.29-3.41)	72 (75)	135 (80)	0.73 (0.38-1.40)
(EBV EA)			0.66 (0.32-1.37)			1.29 (0.34-4.91)			0.77 (0.38-1.56)
(EBV VCA)			0.67 (0.33-1.37)			0.97 (0.28-3.32)			0.74 (0.38-1.42)
EBV EA negative	57 (78)	117 (89)	1.00	10 (44)	18 (51)	1.00	69 (72)	143 (86)	1.00
EBV EA positive	16 (22)	15 (11)	2.27 (1.03-5.01)	13 (57)	17 (49)	2.81 (0.84-9.43)	27 (28)	24 (14)	2.42 (1.17-4.99)
(JCV, BKV)			2.26 (1.01-5.03)			2.91 (0.82-10.38)			2.35 (1.14-4.81)
(EBV VCA)			2.13 (0.87-5.21)			3.27 (0.76-14.16)			2.37 (1.09-5.19)
EBV VCA negative	34 (47)	74 (56)	1.00	12 (52)	26 (74)	1.00	44 (46)	92 (55)	1.00
EBV VCA positive	39 (53)	58 (44)	1.43 (0.80-2.54)	11 (48)	9 (26)	1.48 (0.47-4.70)	52 (54)	75 (45)	1.44 (0.79-2.60)
(JCV, BKV)			1.41 (0.79-2.52)			1.50 (0.47-4.81)			1.42 (0.79-2.56)
(EBV EA)			1.10 (0.58-2.11)			0.76 (0.18-3.18)			1.03 (0.54-1.96)

Abbreviations: EA, early antigen; VCA, viral capsid antigen.
*Repeated measures were available for 47 cases and 94 controls; intraindividual variance accounted for in conditional logistic regression models using a robust sandwich estimation method.
†Adjusted for matching factors, including age at blood draw, sex, and race.

CI, 0.64-1.48; Table 2). Although JCV and BKV seroprevalence were lower in 1989 compared with 1974, similar findings for NHL risk were observed for each CLUE cohort individually (Table 2).

Among cases diagnosed through 1994 (i.e., the cases included in the previous study of EBV), antibodies to EBV capsid antigen were not significantly associated with all NHL subtypes combined (OR, 1.44; 95% CI, 0.79-2.60; Table 2). However, a significantly increased risk of NHL was associated with antibodies to EBV early antigen (OR, 2.42; 95% CI, 1.17-4.99), even after adjustment for antibodies to EBV viral capsid antigen or JCV and BKV (Table 2). Antibodies to JCV and BKV were not associated with NHL in this subgroup of cases with or without adjustment for EBV early antigen and viral capsid antigen (Table 2).

The associations with antibodies for JCV, BKV, or EBV early antigen were not different by NHL subtype (Table 3). No statistically significant associations were observed between JCV or BKV seropositivity and NHL within any hypothetical induction period (Table 3). A 2-fold increased risk of NHL was associated with EBV early antigen seropositivity among cases diagnosed <10 years after blood draw (OR, 2.59; 95% CI, 0.99-6.74), as well as for cases diagnosed 10 to 19 years after blood draw (OR, 2.26; 95% CI, 0.82-6.22; Table 3).

Few participants shifted from polyomavirus antibody seronegative in 1974 to seropositive in 1989 based on the dichotomous absorbance value cutpoint of 0.1 (one case and one control for JCV, three cases and three controls for BKV). Changes in polyomavirus antibodies over a 15-year period are presented graphically for 47 cases and 94 matched controls who donated blood in both 1974 and 1989 (Fig. 1A1-2 and *B1-2*). Although a majority of individuals did not change polyomavirus antibody serostatus over the 15-year period, absolute levels of antibodies to JCV and BKV decreased over time for 70% and 80% of individuals, respectively. Based on visual inspection of the plots, JCV antibody levels seemed to decrease more frequently and at faster rates among controls compared with NHL cases, whereas no clear differences were observed between cases and controls for BKV antibody levels. A corresponding statistically significant inverse trend was observed between the slope of the decline in JCV antibodies and NHL risk (P_{trend} = 0.02), with shallower declines associated with increasing NHL risk (Table 4). An increase in JCV antibody levels was associated with a statistically significant 4-fold risk of NHL compared with those whose levels declined the greatest (OR, 4.59; 95% CI, 1.30-16.25; Table 4). When the analysis was restricted to the 32 cases and 68 controls who were JCV seropositive at one or both of the time points, the same increased risk on NHL was observed for increases in JCV antibodies, although the association was no longer statistically significant (OR, 4.68; 95% CI, 0.78-28.11; P_{trend} = 0.10). Changes in BKV antibody levels were not associated with increased risk of NHL (Table 4).

Among cases diagnosed through 1994, steep increases in EBV early antigen antibodies were observed for NHL cases, but not for controls (Fig. 1C1-2). These steep increases resulted in EBV early antigen antibody seroconversion from 1974 to 1989 among 6 of 12 cases (50%) but only 3 of 19 controls (16%; Table 4). As compared with those who were seronegative at both time points, EBV early antigen seroconversion was associated with a >4-fold risk of subsequent development of NHL (OR, 4.67; 95% CI, 0.83-26.20; Table 4), although this estimate was imprecise due to small sample size. Age at blood donation did not differ across categories of change for each antibody type investigated (Table 4), nor between cases and controls within any given category of change in antibody levels. Among cases, there were no differences in time between blood draw to diagnosis across categories of change in antibody levels (data not shown).

Discussion

No overall associations were observed between JCV or BKV seropositivity and the subsequent development of NHL. The

Table 3. Antibodies to JCV, BKV, and EBV early antigen in relation to subsequent risk of NHL among 140 cases and 340 matched controls, by NHL subtype and time between blood draw and diagnosis

Ab serostatus	JCV			BKV			EBV early antigen*		
	Cases, *n* (%)	Controls, *n* (%)	OR† (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR† (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR† (95% CI)
By NHL subtype									
DLBCL									
Ab negative	34 (37)	63 (34)	1.00	35 (38)	69 (37)	1.00	26 (74)	53 (88)	1.00
Ab positive	59 (63)	123 (66)	0.88 (0.49-1.56)	58 (62)	117 (63)	0.97 (0.52-1.83)	9 (26)	7 (12)	2.21 (0.77-6.36)
Follicular									
Ab negative	15 (30)	33 (33)	1.00	16 (32)	29 (29)	1.00	19 (73)	39 (85)	1.00
Ab positive	35 (70)	77 (77)	1.18 (0.49-2.85)	34 (68)	71 (71)	0.85 (0.34-2.15)	7 (27)	7 (15)	2.56 (0.44-15.00)
Other/unknown‡									
Ab negative	28 (38)	42 (28)	1.00	21 (28)	44 (30)	1.00	24 (69)	51 (84)	1.00
Ab positive	46 (62)	106 (72)	0.63 (0.33-1.22)	53 (72)	104 (70)	1.09 (0.55-2.15)	11 (31)	10 (16)	2.56 (0.90-7.32)
By time between blood draw and diagnosis									
<10 y									
Ab negative	36 (38)	67 (35)	1.00	36 (38)	69 (36)	1.00	28 (64)	58 (81)	1.00
Ab positive	59 (62)	123 (65)	0.88 (0.50-1.52)	59 (62)	121 (64)	0.93 (0.54-1.59)	16 (36)	14 (19)	2.59 (0.99-6.74)
10-19 y									
Ab negative	21 (28)	46 (30)	1.00	21 (28)	43 (28)	1.00	41 (79)	85 (89)	1.00
Ab positive	55 (72)	106 (70)	1.15 (0.61-2.18)	55 (72)	109 (72)	1.04 (0.53-2.07)	11 (21)	10 (11)	2.26 (0.82-6.22)
20-29 y									
Ab negative	20 (43)	25 (27)	1.00	15 (33)	30 (33)	1.00			
Ab positive	26 (57)	67 (73)	0.47 (0.22-1.01)	31 (67)	62 (67)	1.00 (0.45-2.23)			

Abbreviations: DLBCL, diffuse large B-cell lymphoma; Ab, antibody.
*EBV data were not available for cases diagnosed after 1994 (i.e., 20-29 years after blood draw).
†Adjusted for matching factors, including age at blood draw, sex, and race.
‡Includes three cases of T-cell NHL.

most intriguing result of our study was the strong association between an increase in JCV antibodies over time and subsequent risk of NHL among the subset of participants for whom blood was available from both 1974 and 1989. Most people acquire JCV infection as children and remain seropositive throughout adulthood, which is supported by the observation that few of our adult subjects seroconverted to JCV. Therefore, the observed increases in JCV antibody levels among those who were already seropositive may reflect a low-level, chronic reactivation of JCV infection (31). Our results thus suggest the possibility that JCV reactivation occurring in adulthood, and not initial infection, may predispose to NHL.

One mechanism by which JCV reactivation may be involved in NHL development is through infection of B lymphocytes. Reactivation of JCV from the kidney could lead to infection of B lymphocytes (15, 32), whereby the virus could directly contribute to lymphomagenesis. For example, expression of JCV large T antigen in infected lymphocytes might modulate gene transcription and inhibit apoptosis (7, 13). This concept is supported by the previously observed correlation between JCV

Table 4. Change in JCV, BKV, and EBV early antigen antibody levels over time among NHL cases and controls who donated blood to both cohorts: CLUE I in 1974 and CLUE II in 1989

Change in antibody levels from 1974 to 1989	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*	Age in 1974 among	
				Cases: mean (SD)	Controls: mean (SD)
JCV capsid antibodies					
Decline (greatest)†	5 (10.6)	23 (24.5)	1.00 (reference)	53.8 (10.8)	50.4 (14.1)
Decline (intermediate)	11 (23.4)	24 (25.5)	2.65 (0.74-9.58)	51.6 (12.0)	46.5 (9.6)
Decline (least)	13 (27.7)	23 (24.5)	3.01 (0.95-9.49)	48.8 (12.5)	49.0 (12.0)
Increase	18 (38.3)	24 (25.5)	4.59 (1.30-16.25)	44.9 (9.6)	48.4 (9.4)
			P_{trend} = 0.02	P_{ANOVA} = 0.29	P_{ANOVA} = 0.71
BKV capsid antibodies					
Decline (greatest)‡	17 (36.2)	24 (25.5)	1.00 (reference)	47.0 (11.0)	48.0 (12.1)
Decline (intermediate)	9 (19.2)	27 (28.7)	0.49 (0.19-1.25)	53.6 (10.7)	51.0 (12.9)
Decline (least)	11 (23.4)	25 (26.6)	0.63 (0.23-1.73)	48.8 (12.4)	47.8 (10.0)
Increase	10 (21.3)	18 (19.2)	0.92 (0.31-2.72)	46.0 (11.1)	46.6 (9.4)
			P_{trend} = 0.33	P_{ANOVA} = 0.46	P_{ANOVA} = 059
EBV EA antibodies§					
Positive→negative§	1 (8.3)	0 (0.0)	∞ (0-∞)	38.6 (—)	—
Negative→negative	4 (33.3)	13 (68.4)	1.00 (reference)	57.9 (3.3)	50.3 (12.1)
Positive→positive	1 (8.3)	3 (15.8)	0.77 (0.09-6.39)	52.4 (—)	56.9 (4.0)
Negative→positive	6 (50.0)	3 (15.8)	4.67 (0.83-26.20)	48.3 (13.2)	41.0 (2.0)
				P_{ANOVA} = 0.39	P_{ANOVA} = 0.21

*Adjusted for matching factors, including age at blood draw, sex, and race.
†For JCV, the absorbance value cutpoints were 0.33 to 0.64 for greatest decline, 0.086 to 0.32 for intermediate decline, 0.001 to 0.085 for least decline, and >0 to 0.573 for increase.
‡For BKV, the absorbance value cutpoints were 0.223 to 1.04 for greatest decline, 0.072 to 0.222 for intermediate decline, 0.001 to 0.071 for least decline, and >0 to 0.897 for increase.
§EBV antibody data are only available for cases diagnosed through 1994; positive serostatus for EBV early antigen antibodies defined as a reciprocal titer ≥20; no cases shifted from positive to negative for antibodies to EBV viral capsid antigen.

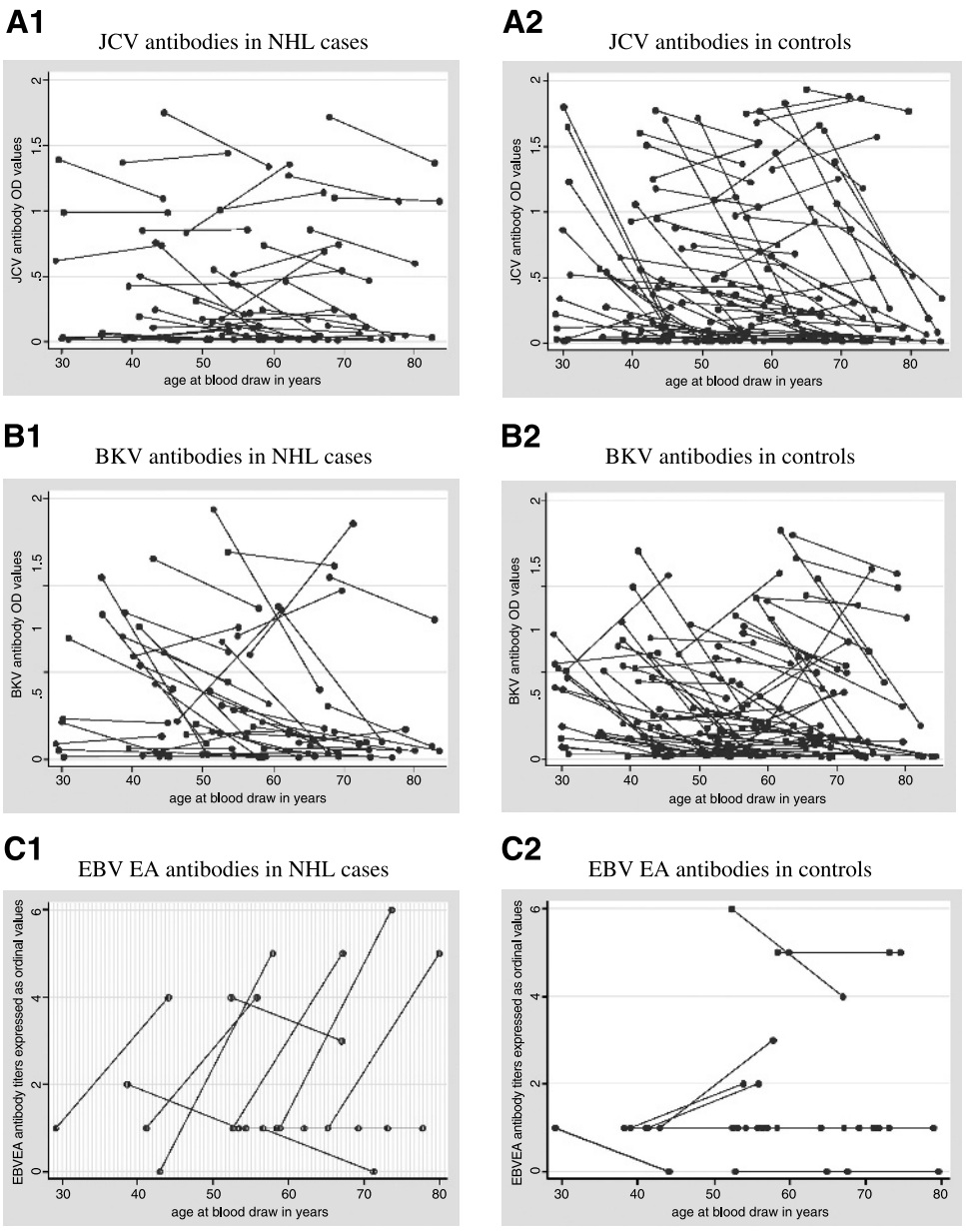

Figure 1. Change in antibody levels between 1975 and 1989 in NHL cases and controls. Intraindividual changes in antibody levels among NHL cases and sex- and age-matched controls are plotted against age at blood draw for JCV (**A1** and **A2**), BKV (**B1** and **B2**), and EBV early antigen (**C1** and **C2**). *X axis,* age at blood draw (in years). *Y axis,* absorbance values for JCV and BKV and ordinal titer values for EBV (reciprocal titers of < 10, 10, 20, 40, 80, 160, and 320 were assigned ordinal values of 0, 1, 2, 3, 4, 5, and 6, respectively).

antibody titers and increased frequency of chromosomal aberrations in peripheral blood lymphocytes (16).

The mechanism underlying the association between changing JCV antibody levels and NHL could also be more indirect. Somewhat surprisingly, we found no association between overall JCV seropositivity and NHL, and Engels et al. (23) observed a statistically significant 30% reduction in NHL risk associated with JCV antibody seropositivity among newly diagnosed population-based NHL cases and matched controls. Results from PCR-based studies to date have been inconsistent. Three studies that were primarily investigations of SV40 in lymphoma tissues detected no JCV or BKV DNA sequences in NHL tissues (20-22). Another laboratory reported JCV and BKV sequences in 5% and 6% of 83 lymphomas, respectively (18). Finally, a report of JCV DNA sequences identified 81% of 27 central nervous system lymphomas analyzed (19). Some investigators have proposed that JCV may contribute to the development of cancer through a ''hit-and-run'' mechanism, whereby the virus is involved in the early stages of carcinogenesis and is eventually lost from malignant cells through subsequent mutations (31). Under this hit-and-run scenario, JCV DNA would not necessarily be present in lymphoma tissues. Alternatively, if JCV infection at early stages of carcinogenesis could be measured by circulating antibodies to the virus, then an association with NHL risk might have been seen among those whose blood was drawn in the critical time period before NHL diagnosis (i.e., induction period). However, our analysis of prospective data by induction period showed no increased risks of NHL associated with JCV antibodies detected in serum collected up to 29 years before diagnosis.

The observed increased risk of NHL associated with rises in JCV antibody levels over time may indicate that JCV reactivation arises due to subtle immune dysregulation, a possible early disease effect. Along these lines, the risk of NHL associated with increasing versus decreasing levels of JCV antibodies was strongest among the 17 cases diagnosed within 5 years of their second blood draw (OR, 4.30; 95% CI, 0.88-21.06), compared with the 20 cases diagnosed 5 to 10 years after their second blood draw (OR, 1.44; 95% CI, 0.44-4.60) and the 10 cases diagnosed >10 years after their second blood draw (OR, 1.00; 95% CI, 0.21-4.81), although none of these associations were statistically significant. Arguing against an early disease effect as the explanation for increasing JCV

antibody levels among cases, we observed no relationship between NHL risk and changes in antibodies to BKV, a closely related polyomavirus. The >2-fold risk of NHL associated with EBV early antigen seropositivity was consistently observed among those diagnosed <10 and 10+ years after blood draw, indicating that EBV reactivation is not an early disease effect. Sample size was too small to investigate whether NHL risk was independently associated with change in JCV antibody levels by EBV early antigen serostatus, although changes in JCV antibody levels over 15 years were not correlated with changes in EBV titers (Spearman correlation coefficient = 0.03, $P = 0.84$).

JCV reactivation of infection could be associated with other viral infections. We previously investigated the association between SV40 antibodies and NHL in this case-control study population (24) and initially observed a statistically significant association. SV40-positive samples were then tested for cross-reactivity with JCV and BKV through competitive inhibition assays. Whereas SV40 antibodies were detected by virus-like particle ELISA in 15% of cases and 10% of controls, the SV40 reactivity of 85% of the SV40 antibody–positive sera was decreased by adsorption with BKV and/or JCV virus-like particles. Antibodies specific for SV40 (not cross-reactive) were identified in only 1.8% of cases and 1.6% of controls, and were not associated with NHL. Based on these previous findings, we had hypothesized that JCV and/or BKV may be the source of these cross-reactive antibodies when we undertook the current study. However, overall seropositivity for JCV and BKV were not associated with increased risk of NHL and adjustment for SV40 cross-reacting antibodies did not change these observed associations (data not shown). Additionally, there was no correlation between antibodies to EBV and SV40. The source of the SV40 cross-reactive antibodies in the previous study is presently unknown. If additional human polyomaviruses capable of inducing the production of antibodies that cross-react with the known human polyomaviruses are identified in the future, then infection with these viruses should be investigated as a potential risk factor for NHL.

Serologic patterns may also be markers for other NHL risk factors, including socioeconomic status or age at infection with childhood viruses. For example, later age at onset of infectious diseases among individuals with no sibling or with one sibling has been associated with increased risk of NHL (33). Similarly, a recent case-control study observed a statistically significant trend in increased risk of NHL associated with birth order, with only children having almost half the risk of developing NHL in adulthood compared with individuals who were fourth-born or later (34). This risk was further reduced among first-born/only children who ever had allergies, suggesting that a Th2-dominant immune response may be protective against NHL (34). If delayed childhood infections lead to an immunologic imbalance in adulthood, which, in turn, results in inefficient immune control of latent viruses with transforming capabilities, then all of these factors may be on the same causal pathway to NHL.

Our findings of increased risk of NHL associated with increasing levels of JCV antibodies should be interpreted with caution because the analysis of changes in polyomavirus antibody levels over time was limited by small sample size. Additionally, no tumor tissues were available from these individuals to investigate the correlation between changes in prediagnostic antibody levels and presence of virus in tissues. However, the unique design of this prospective study enabled us to investigate longitudinal changes in antibody levels within individuals associated with future cancer risk. The lower age-adjusted JCV seroprevalence in CLUE II (1989) versus CLUE I (1974) most likely resulted from the differences in sample types collected because plasma samples (collected in CLUE II) contain antibody at slightly lower concentrations than serum (collected in CLUE I), and the positive cutpoint used in the analysis was derived from serum reference samples. However, the relative differences in seroprevalence between cases and controls would be unaffected by the differences in serum versus plasma, as supported by the similar ORs observed for polyomavirus infection and NHL in both cohorts. Additionally, the observed rises in JCV antibody titers over time and their associations with NHL would be underestimated.

Our findings should be replicated in other cohorts that include repeated measurements over time from each participant. In addition to measuring JCV capsid IgG antibodies, JCV capsid IgA antibodies and JCV DNA shedding in the urine could be used as biomarkers of reactivated of JCV infection. Future research is also needed to identify factors associated with reactivation of latent infections to better evaluate potential confounders in epidemiologic studies and to develop prevention strategies for viral infections determined to be causally related to cancer.

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